



# SEQUENCE LISTING

<110> Lydiate, Derek  
Hannoufa, Abdelali  
Bate, Nicholas  
Hegedus, Dwayne

<120> Repressor Mediated Selection Strategies

<130> 11089.0003.NPUS01

<140> 10/678,490

<141> 2003-10-03

<150> 60/416,369

<151> 2002-10-03

<160> 61

<170> PatentIn version 3.1

<210> 1

<211> 472

<212> DNA

<213> artificial

<220>

<223> Synthetic Ros optimized for plant expression

<400> 1

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ttactgctga tatcgttgct gcttacgttt ctaaccacgt tgttcctggt actgagcttc      120
ctggacttat ctctgatgtt catactgcac tttctggaac atctgctcct gcttctgttg      180
ctgttaacgt tgagaagcag aagcctgctg tttctgttcg taagtctggt caggatgac      240
atatcgtttg tttggagtgt ggtggttctt tcaagtctct caagcgtcac cttactactc      300
atcactctat gactccagag gagtatagag agaagtggga tcttcctggt gattacccta      360
tggttgctcc tgcttacgct gaggctcggt ctcgtctcgc taaggagatg ggtctcggtc      420
agcgtcgtaa ggctaaccgt ccaaaaaaga agcgttaagg ctgagagctc gc              472
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<210> 2

<211> 678

<212> DNA

<213> artificial

<220>

<223> Synthetic Tet optimized for plant expression

<400> 2

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gagcagccta cgttgtactg gcatgttaag aacaagcggg ctttgctcga cgccctcgcg      180
attgagatgt tagacaggca ccatactcac ttctgccttc tcgaagggga gagctggcaa      240
gatttcctcc gtaacaacgc taagtccttc agatgtgctc tcctatcca tcgcgacgga      300
gcaaaagttc atctgggtac acggcctaca gagaaacagt atgagactct cgaaaatcaa      360
ctggcctttc tgtgccaaaca gggtttctca ctagagaatg cgctttacgc actctcagct      420
gtggggcatt ttactcttgg ttgcgttttg gaggatcaag agcatcaagt cgctaaggaa      480
gagagggaaa cacctactac tgatagtatg cgcgcacttc ttcgacaagc catcgaactt      540
tttgatcacc aggggtgcaga gccagccttc ttgttcggcc ttgaattgat catatgcgga      600
ttggaaaagc agcttaaatg tgaatcgggg tctcttaagc caaaaaagaa gcgtaaggtc      660
tgacttaagt gaatcgat                                     678
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<210> 3

<211> 149

<212> PRT

<213> Artificial

<220>

<223> Synthetic Ros

<400> 3

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Met Thr Glu Thr Ala Tyr Gly Asn Ala Gln Asp Leu Leu Val Glu Leu
1           5           10          15
```

```
Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val
          20          25          30
```

```
Thr Glu Leu Pro Gly Leu Ile Ser Asp Val His Thr Ala Leu Ser Gly
          35          40          45
```

```
Thr Ser Ala Pro Ala Ser Val Ala Val Asn Val Glu Lys Gln Lys Pro
          50          55          60
```

Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp His Ile Val Cys Leu  
65 70 75 80

Glu Cys Gly Gly Ser Phe Lys Ser Leu Lys Arg His Leu Thr Thr His  
85 90 95

His Ser Met Thr Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Pro Val  
100 105 110

Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu  
115 120 125

Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Ala Asn Arg Pro Lys  
130 135 140

Lys Lys Arg Lys Val  
145

<210> 4  
<211> 216  
<212> PRT  
<213> Artificial

<220>  
<223> Synthetic Tet

<400> 4

Met Ser Arg Leu Asp Lys Ser Lys Val Ile Asn Ser Ala Leu Glu Leu  
1 5 10 15

Leu Asn Glu Val Gly Ile Glu Gly Leu Thr Thr Arg Lys Leu Ala Gln  
20 25 30

Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr Trp His Val Lys Asn Lys  
35 40 45

Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu Met Leu Asp Arg His His  
50 55 60

Thr His Phe Cys Pro Leu Glu Gly Glu Ser Trp Gln Asp Phe Leu Arg  
65 70 75 80

Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu Leu Ser His Arg Asp Gly  
85 90 95

Ala Lys Val His Leu Gly Thr Arg Pro Thr Glu Lys Gln Tyr Glu Thr  
100 105 110

Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln Gln Gly Phe Ser Leu Glu  
115 120 125

Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly His Phe Thr Leu Gly Cys  
130 135 140

Val Leu Glu Asp Gln Glu His Gln Val Ala Lys Glu Glu Arg Glu Thr  
145 150 155 160

Pro Thr Thr Asp Ser Met Pro Pro Leu Leu Arg Gln Ala Ile Glu Leu  
165 170 175

Phe Asp His Gln Gly Ala Glu Pro Ala Phe Leu Phe Gly Leu Glu Leu  
180 185 190

Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys Cys Glu Ser Gly Ser Leu  
195 200 205

Lys Pro Lys Lys Lys Arg Lys Val  
210 215

<210> 5  
<211> 24  
<212> DNA  
<213> Artificial

<220>  
<223> Actin2 promoter sense primer

<400> 5

aagcttatgt atgcaagagt cagc

24

<210> 6  
<211> 24  
<212> DNA  
<213> Artificial

<220>  
<223> Actin2 promoter anti-sense primer

<400> 6

ttgactagta tcagcctcag ccat

24

<210> 7  
<211> 27  
<212> DNA  
<213> Artificial

<220>  
<223> Ros sense primer

<400> 7

gcggatccga tgacggaaac tgcatac

27

<210> 8  
<211> 25  
<212> DNA  
<213> Artificial

<220>  
<223> Ros anti-sense primer

<400> 8  
gcaagcttca acggttcgcc ttgcg

25

<210> 9  
<211> 36  
<212> DNA  
<213> Artificial

<220>  
<223> iaaH sense primer

<400> 9

tgcggatgca taagcttgct gacattgcta gaaaag

36

<210> 10  
<211> 26  
<212> DNA  
<213> Artificial

<220>  
<223> iaaH anti-sense primer

<400> 10

cggggacccct ttcagggcca tttcag

26

<210> 11

<211> 43

<212> DNA

<213> Artificial

<220>

<223> Tet-FI primer

<400> 11

gatcactcta tcagtgatag agtgaactct atcagtgata gag

43

<210> 12

<211> 41

<212> DNA

<213> Artificial

<220>

<223> Tet-RI primer

<400> 12

cgctctatca ctgatagagt tcaactctatc actgatagag t

41

<210> 13

<211> 26

<212> DNA

<213> Artificial

<220>

<223> iaaH ORF sense primer

<400> 13

gctctagaat ggtgccatt acctcg

26

<210> 14

<211> 26

<212> DNA

<213> Artificial

<220>

<223> iaaH ORF anti-sense primer

<400> 14

gcgagctcaw atggcttytt cyaatg 26

<210> 15  
<211> 59  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OP1

<400> 15

gatactatat ttcaatttta ttgtaataata gctatatttc aattttattg taatataat 59

<210> 16  
<211> 57  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OP2

<400> 16

cgattatatt acaataaaat tgaaatatag ctatattaca ataaaattga aatatag 57

<210> 17  
<211> 25  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 17

tatatttcaa ttttattgta atata 25

<210> 18  
<211> 27  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 18

tataattaaa atattaactg tcgcatt 27

<210> 19  
<211> 429  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 19

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atgacggaaa ctgcatacgg taacgcccag gatctgctgg tcgaactgac ggcggatatt      60
gtggctgcct atgttagcaa ccacgtcggt ccggttaactg agcttcccgg ccttatttcg    120
gatgttcata cggcactcag cggaacatcg gcaccggcat cggtggcggg caatgttgaa    180
aagcagaagc ctgctgtgtc ggttcgcaag tcggttcagg acgatcatat cgtctgtttg    240
gaatgtggtg gctcgttcaa gtcgctcaaa cgccacctga cgacgcatac cagcatgacg    300
ccggaagaat atcgcgaaaa atgggatctg ccggtcgatt atccgatggt tgctcccggc    360
tatgccgaag cccgttcgcg gtcgccaag gaaatgggtc tcggtcagcg ccgcaaggcg    420
aaccgttga                                     429
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<210> 20

<211> 624

<212> DNA

<213> *escherichia coli*

<400> 20

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atgtctagat tagataaaag taaagtgatt aacagcgcac tagagctgct taatgaggtc      60
ggaatcgaag gcctaacaac ccgtaaactt gcgcagaagc tcggggtaga gcagcctaca    120
ttgtattggc atgtaaaaaa taagcggggc ctgctcgacg cgttagccat tgagatgtta    180
gataggcacc atactcactt ttgcccttta gaaggggaaa gctggcaaga ttttttacgt    240
aataacgcta aaagttttag atgtgcttta ctaagtcata gcgatggagc aaaagtacat    300
ttaggtacac ggcctacaga aaaacagtat gaaactctcg aaaatcaatt agccttttta    360
tgccaacaag gtttttcact agagaatgca ttatatgcac tcagcgctgt ggggcatttt    420
actttagggt gcgtattgga agatcaagag catcaagtcg ctaaagaaga aagggaacaa    480
cctactactg atagtatgcc gccattatta cgacaagcta tcgaattatt tgatcaccaa    540
ggtgcagagc cagccttctt attcggcctt gaattgatca tatgoggatt agaaaaacaa    600
cttaaattgt aaagtgggtc ttaa                                     624
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<210> 21

<211> 142

<212> PRT

<213> *Agrobacterium tumefaciens*



<400> 21

Met Thr Glu Thr Ala Tyr Gly Asn Ala Gln Asp Leu Leu Val Glu Leu  
1 5 10 15

Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val  
20 25 30

Thr Glu Leu Pro Gly Leu Ile Ser Asp Val His Thr Ala Leu Ser Gly  
35 40 45

Thr Ser Ala Pro Ala Ser Val Ala Val Asn Val Glu Lys Gln Lys Pro  
50 55 60

Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp His Ile Val Cys Leu  
65 70 75 80

Glu Cys Gly Gly Ser Phe Lys Ser Leu Lys Arg His Leu Thr Thr His  
85 90 95

His Ser Met Thr Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Pro Val  
100 105 110

Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu  
115 120 125

Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Ala Asn Arg  
130 135 140

<210> 22

<211> 207

<212> PRT

<213> Escherichia coli

<400> 22

Met Ser Arg Leu Asp Lys Ser Lys Val Ile Asn Ser Ala Leu Glu Leu  
1 5 10 15

Leu Asn Glu Val Gly Ile Glu Gly Leu Thr Thr Arg Lys Leu Ala Gln  
20 25 30

Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr Trp His Val Lys Asn Lys  
35 40 45

Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu Met Leu Asp Arg His His  
50 55 60

Thr His Phe Cys Pro Leu Glu Gly Glu Ser Trp Gln Asp Phe Leu Arg  
65 70 75 80

Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu Leu Ser His Arg Asp Gly  
85 90 95

Ala Lys Val His Leu Gly Thr Arg Pro Thr Glu Lys Gln Tyr Glu Thr  
100 105 110

Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln Gln Gly Phe Ser Leu Glu  
115 120 125

Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly His Phe Thr Leu Gly Cys  
130 135 140

Val Leu Glu Asp Gln Glu His Gln Val Ala Lys Glu Glu Arg Glu Thr  
145 150 155 160

Pro Thr Thr Asp Ser Met Pro Pro Leu Leu Arg Gln Ala Ile Glu Leu  
165 170 175

Phe Asp His Gln Gly Ala Glu Pro Ala Phe Leu Phe Gly Leu Glu Leu  
180 185 190

Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys Cys Glu Ser Gly Ser  
195 200 205

<210> 23  
<211> 10  
<212> DNA  
<213> Artificial

<220>  
<223> Consensus Ros operator sequence

<400> 23

watdhwkmar

10

<210> 24  
<211> 7  
<212> PRT  
<213> SV40

<400> 24

Pro Lys Lys Lys Arg Lys Val  
1 5

<210> 25  
<211> 109  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPDS

<400> 25

atctccactg acgtaaggga tgacgcacaa tcccactatc cttegcaaga cccttcctct 60

atataatata tttcaatttt attgtaatat aacacggggg actctagag 109

<210> 26  
<211> 113  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPDA

<400> 26

gatcctctag agtcccccggt gttatattac aataaaattg aaatatatta tatagaggaa 60

gggtcttgcg aaggatagtg ggattgtgcg tcatccctta cgtcagtgga gat 113

<210> 27  
<211> 138  
<212> DNA  
<213> Artificial

<220>  
<223> p74-315 sequence from EcoRV to ATG of GUS

<400> 27

gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 60  
tctatataat atatttcaat tttattgtaa tataacacgg gggactctag aggatccccg 120  
ggtggtcagt cccttatg 138

<210> 28  
<211> 107  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPUS

<400> 28

atctccactg acgtaaggga tgacgcacaa tctatatttc aattttattg taatatacta 60  
tataaggaag ttcatttcat ttggagagaa cacgggggac tctagag 107

<210> 29  
<211> 111  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPUA

<400> 29

gacctctag agtcccccggt gttctctcca aatgaaatga acttccttat atagtatatt 60  
acaataaaat tgaaatatag attgtgcgtc atcccttacg tcagtggaga t 111

<210> 30  
<211> 136  
<212> DNA  
<213> Artificial

<220>  
<223> p74-316 sequence from EcoRV to ATG of GUS

<400> 30

gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatata 60  
ctatataagg aagttcattt catttggaga gaacacgggg gactctagag gatccccggg 120  
tggtcagtc cttatg 136

<210> 31

<211> 108  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPPS

<400> 31

atctccactg acgtaaggga tgacgcacaa tctatatttc aattttattg taatatacta 60  
tataatatat ttcaatttta ttgtaatata acacggggga ctctagag 108

<210> 32  
<211> 112  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPPA

<400> 32

gacccctctag agtcccccggt gttatattac aataaaattg aaatatatta tatagtatat 60  
tacaataaaa ttgaaatata gattgtgcgt catcccttac gtcagtggag at 112

<210> 33  
<211> 137  
<212> DNA  
<213> Artificial

<220>  
<223> p74-309sequence from EcoRV to ATG of GUS

<400> 33

gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatata 60  
ctatataata tatttcaatt ttattgtaat ataacacggg ggactctaga ggatccccgg 120  
gtggtcagtc ccttatg 137

<210> 34  
<211> 237  
<212> DNA  
<213> Artificial

<220>  
<223> p74-118 sequence from EcoRV to ATG of GUS

<400> 34

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gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 60
tctatataat atatttcaat tttattgtaa tataacacgg gggactctag aggatcctat 120
atttcaattt tattgtaata tagctatatt tcaattttat tgtaataata tcgatttcga 180
acccggggta ccgaattcct cgagtctaga ggatccccgg gtggtcagtc ccttatg 237
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<210> 35

<211> 235

<212> DNA

<213> Artificial

<220>

<223> p 74-117 sequence from EcoRV to ATG of GUS

<400> 35

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gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatata 60
ctatataagg aagttcattt catttggaga gaacacgggg gactctagag gacccctat 120
ttcaatttta ttgtaatata gctatatttc aattttattg taatataatc gatttcgaac 180
ccgggggtacc gaattcctcg agtctagagg atccccgggt ggtcagtcct ttatg 235
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<210> 36

<211> 16

<212> PRT

<213> Arabidopsis

<400> 36

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Arg Ile Glu Asn Thr Thr Asn Arg Gln Val Thr Phe Cys Lys Arg Arg
1           5           10           15
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<210> 37

<211> 18

<212> PRT

<213> Tobacco

<400> 37

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Arg Arg Leu Ala Gln Asn Arg Glu Ala Ala Arg Lys Ser Arg Ile Arg
1           5           10           15
```

Lys Lys

<210> 38  
<211> 20  
<212> PRT  
<213> Tobacco

<400> 38

Lys Lys Arg Ala Arg Leu Val Asn Arg Glu Ser Ala Gln Leu Ser Arg  
1 5 10 15

Gln Arg Lys Lys  
20

<210> 39  
<211> 18  
<212> PRT  
<213> Maize

<400> 39

Arg Lys Arg Lys Glu Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Tyr  
1 5 10 15

Arg Lys

<210> 40  
<211> 45  
<212> PRT  
<213> Potyvirus

<220>  
<221> MISC\_FEATURE  
<222> (11)..(42)  
<223> where Xaa is any amino acid

<400> 40

Lys Lys Asn Gln Lys His Lys Leu Lys Met Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Arg Lys

35

40

45

<210> 41  
<211> 17  
<212> PRT  
<213> Xenopus

<400> 41

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys  
1 5 10 15

Ile

<210> 42  
<211> 17  
<212> PRT  
<213> Xenopus

<400> 42

Lys Arg Ile Ala Pro Asp Ser Ala Ser Lys Val Pro Arg Lys Lys Thr  
1 5 10 15

Arg

<210> 43  
<211> 17  
<212> PRT  
<213> Xenopus

<400> 43

Lys Arg Lys Thr Glu Glu Glu Ser Pro Leu Lys Asp Lys Asp Ala Lys  
1 5 10 15

Lys

<210> 44  
<211> 17  
<212> PRT  
<213> Rat



<400> 44

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 45

<211> 17

<212> PRT

<213> Human

<400> 45

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 46

<211> 17

<212> PRT

<213> Human

<400> 46

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 47

<211> 17

<212> PRT

<213> Chicken

<400> 47

Arg Lys Cys Cys Gln Ala Gly Met Val Leu Gly Gly Arg Lys Phe Lys  
1 5 10 15

Lys

<210> 48  
<211> 17  
<212> PRT  
<213> Human

<400> 48

Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg Lys Ile Lys  
1 5 10 15

Lys

<210> 49  
<211> 17  
<212> PRT  
<213> Chicken

<400> 49

Arg Arg Cys Phe Glu Val Arg Val Cys Ala Cys Pro Gly Arg Asp Arg  
1 5 10 15

Lys

<210> 50  
<211> 236  
<212> DNA  
<213> Artificial

<220>  
<223> p74-114 sequence from EcoRV to ATG of GUS

<400> 50  
gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatat 60  
ctatataata tatttcaatt ttattgtaat ataacacggg ggactctaga ggatcctata 120  
tttcaatttt attgtaatat agctatatatt caattttatt gtaatatataat cgatttcgaa 180  
cccggggtac cgaattcctc gagtctagag gatccccggg tggtcagtcc cttatg 236

<210> 51  
<211> 33  
<212> DNA  
<213> Artificial

<220>  
<223> synRos forward primer

<400> 51

gcggatccat gactgagact gcttacggta acg

33

<210> 52  
<211> 29  
<212> DNA  
<213> Artificial

<220>  
<223> synRos reverse primer

<400> 52

gcgagctcga ccttacgctt cttttttgg

29

<210> 53  
<211> 26  
<212> DNA  
<213> Artificial

<220>  
<223> wtRos forward primer

<400> 53

cgggatccat gacggaaact gcatac

26

<210> 54  
<211> 24  
<212> DNA  
<213> Artificial

<220>  
<223> wtRos reverse primer

<400> 54

gcgagctcac ggttcgctt gcgg

24

<210> 55  
<211> 108  
<212> DNA  
<213> Artificial

<220>

<223> Ros oligonucleotide for Southwestern

<400> 55

atctccactg acgtaaggga tgacgcacaa tctatatttc aattttattg taatatacta 60

tataatatat ttcaatttta ttgtaatata acacggggga ctctagag 108

<210> 56

<211> 43

<212> DNA

<213> Artificial

<220>

<223> Tet oligonucleotide for Southwestern

<400> 56

gatcactcta tcagtgatag agtgaactct atcagtgata gag 43

<210> 57

<211> 10

<212> DNA

<213> Agrobacterium tumefaciens

<400> 57

tatatttcaa 10

<210> 58

<211> 10

<212> DNA

<213> Agrobacterium tumefaciens

<400> 58

tatattacaa 10

<210> 59

<211> 10

<212> DNA

<213> Agrobacterium tumefaciens

<400> 59

tataattaaa 10

<210> 60

<211> 10

<212> DNA  
<213> Agrobacterium tumefaciens

<400> 60

aatgcgacag

10

<210> 61  
<211> 10  
<212> DNA  
<213> Artificial

<220>  
<223> Ros operator sequence (1)

<400> 61

tatahttcaa

10